STUDIES ON CONTROL OF PLANT NEMATODES BY ORGANIC AMENDMENTS

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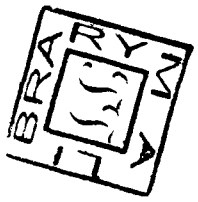

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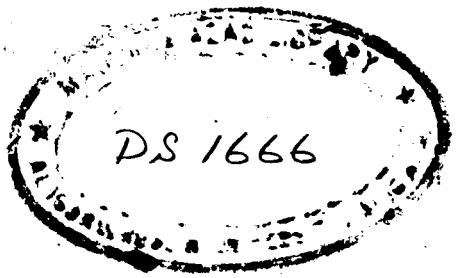
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Dedicated to my respectable father.

LATE Mr. MOHD. YAQUB, *whose*

blessings and inspiration

continue to sustain

me amidst

vicissitudes

of

life

A C K N O W L E D G E M E N T

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I am greatly pleased to set down for remembrance my estimation to my laboratory colleagues.

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M. Imran

(MOHD. IMRAN)

C O N T E N T S

INTRODUCTION

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It has now firmly been established that nematodes are a bane in crop production. Although at the present state of knowledge of plant nematology, no precise data of the amount of damage caused by nematodes to various crops are available, but approximate losses have been expressed in terms of money in some countries. In U.S.A. alone, the estimated annual loss in cultivated crops due to plant nematodes ranges from $250,000,000 (Hutchinson et al., 1961) to $500,000,000 (Cairns, 1955). Later, Taylor (1967) based on his observation of U.S.D.A. estimations on 16 vegetable crops, reported an yearly loss of $372,335,00. The annual loss of potato in England due to golden nematode, Heterodera rostochiensis Woll, 1923, alone is estimated to be as high as £2,000,000 (Southey and Samuel,1954). Recently, a committee of the society of nematologists had estimated that vegetable crops suffer an 11.00 per cent annual loss due to nematodes (Feldmesser et al., 1971). While dealing with the details, the same committee reported that annual losses due to nematodes on field crops are of the order of $1,038,374,300; on fruit and nut crops, $225,145,900; on vegetable crops, $266,989,100 and on ornamental crops, $59,817,634.

In India Krishnappa (1985) has summarized the crop losses in terms of money or percentage of area infected due to the plant parasitic nematode. Van Berkum and Seshadri(1970) reported the annual loss of $10 million from "ear cockle"

disease caused by Anguina tritici in wheat and $ 8 million due to 'Molya disease' caused by Heterodera avenae in the province of Rajasthan. Paruthi and Bhatti (1985) reported a loss of 2.85% in yield of wheat due to Anguina tritici. In another report Handa et al., (1985) estimated the losses in barley due to Heterodera avenae to the tune of Rs. 1687-5911 per hectare. In India perhaps none of our cultivated crops is as susceptible to root-knot nematodes as the vegetable crops and an infestation upto 85% has been observed on tomato, egg plant, okra, cucurbits, potato, tobacco, papaya, jute, cotton and groundnut etc. In certain regions of Himachal Pradesh and Ranchi (Bihar), it has become most uneconomic to grow potatoes and other vegetable crops because of root-knot nematodes (Prasad, 1964).

Similarily, Khan et al., (1966) have pointed out that root-knot nematode is the most important nematode pest on vegetables.

Main aim in the control of nematodes is to bring down the number of nematodes to a level at which no significant damage is caused to plants, various methods have been adopted for the control of nematodes which are not very different from those employed for other parasitic organisms. They traditionally fall in four broad categories, viz., physical, chemical, biological and cultural methods. Where physical and chemical measures of control of nematodes have some limitations or are not frequently in use because of one or the other reasons,

cultural and land management practices have given satisfactory control. The various methods under this category include fallow, flooding, growing cover crops, changing time of planting; removal and destruction of infected plants; sowing trap and antagonistic crops; nutrition and general care of host; sanitation including use of nematode free planting stalk and crop rotations (Nusbaum and Ferris, 1973); use of resistant varieties (Moor, 1960 and Howard, 1965), and application of organic matter (Oostenbrink, 1960, Brown, 1965; Khan 1969; Singh and Sitaramaiah, 1970, Sayre, 1971). Despite many reports on the success of organic amendments for nematode control, still many plants, which are of lesser economic importance and are weeds, need be studied for their efficacy in nematode control. Where such different methods are integrated together more effective control of nematodes has been observed. This comes under the category of Integrated pest management. This concept of IPM was first given by entomologist for resolving the problem of resistance against pesticides. It has now been successfully used in plant pathology. Very few studies have been made in plant nematology (Bird 1987) and therefore it is difficult to make generalisation which combination(s) of the methods would be very successful for nematode control. Hence, in the present studies an attempt will be made to determine the effect of soil amendment with plant parts known for antimicrobial properties and not studied so far on nematodes with special reference to root-knot nematode and to integrate these with other organic amendments/nematicides to have maximum reduction of nematodes.

REVIEW OF LITERATURE

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Considerable work has been carried out on the effect of different organic amendments on phytoparasitic nematodes and in most of the cases the organic matters have been found to have suppressive effect on nematode disease development. The organic matters used were mostly of plant origin or waste farm products, however, their impact on improving plant growth and crop yield was variable.

Main findings however can be summarised as follows:-

Green matter (manure) soil amendment

When chopped leaves of pine-apple were applied to the soil at the rate of 50-200 tons/acre, significant reduction in root-knot was observed by Linford et al., (1938). While Duddington and Duthoit (1960) noticed high reduction in the population of Heterodera avenae when chopped cabbage leaves were incorporated into the infested plots. Hutchinson et al., (1960) managed the population of Hoplolaimus tylenchiformis and Pratylenchus penetrans by allowing pumpkin pieces to rot in the field. Chopped leaves of many plants significantly reduced the root-knot nematode (Hameed 1970, Mankau 1962, 1968 , Haseeb et al., 1978, 1984b).

When chopped floral plant parts were incorporated in nematode infested soil, the growth of aubergines was increased

with the reduction in the population of plant parasitic nematodes. The highest reduction in population was observed when soil was amended with flowers of Iresine herbstii (61.96%) followed by Syzygium cuminii (61.28%). However highest increase in plant growth was observed when soil was amended with chopped leaves of Ricinus communis (Haseeb & Alam 1984, Haseeb et al., 1984). Alam (1987) incorporated chopped plant leaves into naturally infested soil and observed that it effectively suppressed growth of tomato cv. marglobe. Taylor and Murant (1966) observed that the population of Longidorus elongatus was reduced by incorporating raspberry canes to the soil. Alam (1986) used chopped shoots of some weeds and noticed significant reduction in the population of Meloidogyne incognita and Tylenchorhynchus brassicae on egg plant cv. PPL. The nematode population was reduced significantly by adding green manure to the soil (Gour and Prasad, 1970, Sitaramaiah 1978). Green leaves of many plants viz. Azadirachta indica, Melia azedarach, Cassia fistula, C. occidentalis, Crotalaria juncea and Sesbania aculeata significantly reduced the population of Meloidogyne javanica and root-knot development (Singh 1965, Singh and Sitaramaiah 1967, 1973, Zaiyd 1977, Gupta and Ram 1981, Ram and Gupta 1980, 1982). There has been gradual reduction in the population of Meloidogyne hapla by incorporating various parts of Crotalaria sp. and marigold in soil (Yuhara, 1971); of Tylenchorhynchus dubius and Hoplolaimus sp. by the addition of homogenized leaves of corn, tomato and

hemlock. (Miller, 1978; Miller et al., 1973b). Neem leaves also resulted in satisfactory control of root-knot nematodes (Zaiyd, 1977). In a similar study, Haseeb et al., (1978) noted that chopped leaves of Calotropis procera reduced the nematode population.

In order to manage population of Pratylenchus penetrans and Meloidogyne incognita, green manure and some poor host alternative crops were used during summer; groundnut, marigold, sorghum or water melon were grown in four replicates in the summer of the first year and in the subsequent season all plots were planted with japanese radish (winter), water melon (summer) followed by Japanese radish again (winter). In plots where water melon was grown, population density of P. penetrans increased gradually and that of Meloidogyne incognita increased rapidly. The P. penetrans density was greater after sorghum cultivation than after water melon and the sorghum was severly damaged. Both P. penetrans and Meloidogyne incognita populations decreased remarkably during cultivation of marigold and groundnut in the first cropping but both had increased in the second cropping season. In the pot experiment Asparagus was the most effective in reducing P. penetrans while Crotalaria sp. was not found effective; sweet potato, star cucumber, upland rice and other crops were, however, intermediate in effectiveness (Chikaoka 1982).

Mian and Rodriguez-Kabana (1982 b,c) pointed out that spent coffee grinds, Crotalaria, Kudzu (Puernaria lobata) or

ramie (Boehmeria nivea) hays applied at 1% (w/w) were most effective in reducing root-knot galling caused by Meloidogyne arenaria on Cucurbita pepo. Tagetes erecta, T. patula and Calendulla officinalis were found effective as green crop manure for controlling stem nematode infection on strawberry when applied nine months before planting (Andreeva 1975). Later oats, mustard, rye and maize were found effective in reducing the population of nematode when used as green manure crops (Andreeva 1983). Radish var. siletina, mustard var. Maxi and phacelia var. Angelia were used as green manure crops after winter, barley and the population of sugarbeet nematode was determined. The susceptible variety of radish i.e. siletina gave a nematode multiplication rate of 3.71 in the upper soil layer but at a depth of 30-45 cm it was 1.27, when the resistant varieties such as Pegletta and Maxi were sown, the rate of multiplication was 0.59 and 0.54 respectively in the upper soil layer, 0.30 and 0.25 at 30 to 45 cm depth thus indicating a reduction with increasing depth and with resistant varieties. This reduction in nematode numbers in the deeper layers is of great significance as it prevented the subsequent active or passive transference of larvae to the upper soil layer (Steudel 1985).

Farm yard manure

Vander Laan (1956) reported that population of Heterodera rostochiensis was reduced when soil was amended with farmyard manure and compost in potato fields. Inhibition

in the population of plant parasitic nematodes with farmyard manures together with better yield of crops was also reported by Duddington et al., (1961), Patel and Desai (1964), Hams and Wilkins (1964), Nollen (1964), VLK (1972, 1973 and Resck et al., (1982). Reduction in gall index on tomato caused by Meloidogyne incognita was reported as the amount of compost increased in the soil (Guiran and Bonnel, 1979). VLK and Holubcova (1982) pointed out that when leaf litter, farmyard manure and maize straw were added to soil containing onion residues contaminated with Ditylenchus dipsaci there was a reduction of 37.9% in nematode population over 3 months and a 30.2% drop after 16 months. Chindo and Khan (1986) applied poultry manure to the soil infested with Meloidogyne incognita in pot and field prior to planting tomato seedlings and observed that the growth and fruit yield increased while nematode damage decreased with increasing level of manure. Derrico and Dimaio (1980) found that addition of dried poultry faeces, dried poultry manure, composted oil cake, composted poultry manure in the field of tomatoes significantly reduced infestation of Meloidogyne incognita. Undecomposed or partially decomposed organic matter had the greatest effect in reducing nematode population.

Dried waste as soil amendment

Roy (1976, 1979) observed that the root-knot on jute and rice caused by Meloidogyne javanica was reduced by amending the soil with decaffeinated tea waste and water hyacinth compost. Contradictory reports indicating failure of composted materials to control root-knot and other nematodes have also been published (Wager, 1945; Alam, 1976). Heald and Burton (1968), Saka (1978) and Hornick et al., (1984) pointed out that organic nitrogen in the form of activated sewage sludge was more effective than ammonium nitrate for reducing the population of Belonolaimus longicaudatus and Hypsoperine graminis in turf grass. Coosemans(1982) noticed that addition of 10% fermented household waste to the soil infested with Meloidogyne hapla resulted in a minimal amount of root-knot together with maximum microbiological activity. Higher amount of waste led to increased damage due to Meloidogyne hapla, however, addition of upto 20% ground cocoa bean waste decreased the amount of root-knot. Bora and Phukan (1983) observed that all the four soil amendments: poultry manure, sawdust, decaffeinated tea waste and mustard oil cake gave significant reduction of Meloidogyne incognita on jute. The efficacy was related to dose rate, with 2.4% being more effective than 1.2 and 0.6% w/w. Mustard oil cake was the most effective but was also phytotoxic. Sawdust was more effective than tea waste. The poultry manure at the lowest dose was the least effective. Sawdust and to a lesser extent tea waste, had the best effect on plant height and

the dry and fresh weight of shoots and roots. Sharma et al., (1985) on applying leaf powder of certain plants to soil noticed considerable reduction in the population of Meloidogyne incognita. Paddy husk was also found highly effective in reducing the infection of Meloidogyne javanica on various crop plants (Chaudhary 1981). Cocoapod husks, cassava peelings or rice husk have also been used successfully as soil amendments for the control of Meloidogyne incognita on Vigna unguiculata. Egunjobi and Olaitan (1985, 1986), Johnson (1959, 1962, 1963, 1971, 1972, 1974) and Johnson et al., (1967) used successfully a number of dried residues, such as oat straw, alfalfa, orchard grass, fescue and flax residues to reduce root-knot in tomato. Wheat straw (Gour and Prasad, 1970) and rice husk (Sikora et al., 1973) were also used to reduce the root-knot nematode population to a varying degree. Tomerlin and Smart (1969) reported that the population of Belonolaimus longicaudatus and other plant parasitic nematodes were reduced by the application of rice straw at the rate of 9.0 or 17.9 t/ha cereal straw, oat straw, buck wheat Hull, cocobean hull and timothy hay were found capable of reducing the population of Pratylenchus penetrans (Walker and Specht 1967). Mankau (1962) and Mankau and Minteer (1962) used oat hay, alfalfa hay, alfalfa pallets, cotton waste, sugar beet pulp to reduce the population of Tylenchulus semipenetrans. Sitaramaiah (1978) noted that dry straw and wood sawdust can reduce the population of plant parasitic nematodes to a varying degree. Singh et al.,

(1967) and Singh and Sitaramaiah (1967, 1971 a,b) while applying sawdust in okra and tomato, and Srivastava et al., (1971) in tomato and egg plant observed significant reduction in the intensity of root-knot on these crops. Alam (1984) suggested that high C/N ratio was responsible for nematode control by the application of wood sawdust.Singh et al., (1986) used sawdust as soil amendment for reducing population of plant nematodes. The sawdust was found phytotoxic but the damage was mitigated by the addition of nitrogen in the form of oil cakes, cow dung, leaf mould and urea. Moreover the plants which were grown in sawdust/oil cakes amended soil had higher concentration of phenolics which may be one of the reasons for poor nematode multiplication and better growth of the plants. Contrarily Ponchillia (1972) has pointed out that Xiphinema americanum was able to survive at higher organic matter contents in soil. Mukhopadhyaya et al., (1980) concluded that corm powder of Typhonium tribolatum mixed with soil at 0.3 and 0.6 g/kg soil and DD at 150 µg/kg soil reduced the numbers of galls on roots and increased growth of plants infested with Meloidogyne incognita. Schauer-Blume (1988) investigated the effect of crushed seeds of neem (Azadirachta indica) and residues of a MCOH/MTB seed kernel extraction (AZT - residue) on Pratylenchus penetrans. All the neem amendments resulted in marked reduction in numbers of P. penetrans in roots of winter wheat. In the oil cake and AZT-residue applications the nematode population was reduced to 7% and 20% of the control respectively.

3. Chitin and other soil amendments

Chitin:

Chitin and cellulose in pure or crude form (Mankau 1963; Mankau and Das 1969, 1974; Muzzarelli, 1977; Saka, 1978; Mian et al., 1982; Godoy et al., 1983; Rodriguez Kabana et al., 1983, 1984; Culbreath et al., 1985). Chopped paper pine and tree bark (Miller and Edington, 1962; Miller and Wihrheim, 1966; Miller et al., 1968), chopped pine or spruce bark (Hoestra and Harshagen 1981); cellulose (Miller et al., 1973 a, Singh and Sitaramaiah, 1970) have been used to control a variety of nematodes.

Spiegel et al., (1987) treated bean (Phaseolus vulgaris) and tomato seedlings with different amounts of chitin and ammonia and inoculated with Meloidogyne javanica. The infection due to nematode decreased with increasing amount of ammonia, while chitin caused relatively small amount of reduction in gall formation. In the first cycle, galling index of the chitin treated plant was similar to that of untreated plants in the ammonia exposed, while in non-ammonia exposed soil, chitin reduced galling. In the second cycle, chitin reduced galling in both ammonia exposed and un-exposed soils. Differences in fresh shoot weight between nematode infected and nematode free plants amended with chitin were greater under un-exposed than ammonia exposed soil.

Meals

Alam et al., (1977b) found that the population build up

of Hoplolaimus indicus, Helicotylenchus indicus, Rotylenchulus reniformis, Tylenchorhynchus brassicae, Tylenchus filiformis and Meloidogyne incognita was effectively suppressed by the application of bone meal in twelve different crops. Morgan and Tarjan (1981) concluded that when plants of centipedegrass predominantly infected with Belonolaimus longicaudatus, Hemicycliophora sp. and Criconemoides sp. were treated with three kelp products, kelp meal, Maxicrop seaweed extracts and sea born liquid sea weed, Maxicrop and sea born liquid, considerably reduced the numbers of B. longicaudatus, but sea born liquid remaining effective for longer duration. However, there was no significant response on other nematodes.

Oil cakes

Oil seed cakes/meals probably got greatest favour by a large number of workers particularly from India, partly due to (a) its effectiveness in reducing insect and nematode pest in soil; (b) its availability in bulk together with the ease with which it could be applied. Reduction in root-knot injury in tomato, eggplant, okra, and chilli was observed after incorporating the oil cakes to the infested soil (Singh 1965; Singh and Sitaramaiah, 1966, 1971a, 1973; Goswami and Swarup 1971, Srivastava et al., 1971; Gowda and Shetty, 1973; Alam et al., 1980; Jagdale et al., 1985). Singh and Sitaramaiah (1966) observed that the root-knot on tomatoes, grown after the okra crop can be checked by the residual effect of oil-cakes in the same field without further amendments.

Suppression of root-knot in soil amended with tung nut meal (Aleuritis fordii) was observed by Gill (1952); and that of Pratylenchus penetrans with corn meal and soyabean meal by Walker (1969), Walker et al., 1967 and Walker and Specht (1967). Soil amendment with oil cakes also showed significant reduction in the population of nematode attacking moong (Mishra and Prasad 1974), wheat (Gour and Prasad, 1970; Mishra and Prasad, 1974), and wheat followed by moong and maize (Prasad et al., 1972) and Paddy (Mathur and Prasad, 1973). Ismail et al., (1976) reported that all the oil cakes tested were equally effective in reducing the population of a number of plant parasitic nematodes on different varieties of tomato. Alam et al., (1977c) noted that oil cakes of castor, mustard, neem and groundnut suppressed the population of Hoplolaimus inducus, Tylenchorhynchus brassicae, Tylenchus filiformis and Meloidogyne incognita around tomato, potato and radish. The beneficial effect of these treatments was observed even after a lapse of six months when corn, bottle gourd and sannhemp were grown in the following season. Alam (1976) demonstrated that oil cakes were equally effective in both winter, summer seasons of India and also in two different soil types with high organic content (pH 8.4) with less organic content (pH 7.7).

Oil cakes of cotton seed and peanut reduced root galling caused by Meloidogyne arenaria and also stimulated plant growth of Cucurbita pepo (Mian and Rodriguez-Kabana

1982a). Soil treatment with oil cakes was very effective in reducing Meloidogyne exigua on coffee (Moraes 1976). Jaenh and Lambert (1983) concluded that when coffee (cv. Mundo Novo) seedlings were treated with the extract of castor oil seed cake and planted in the field, the population of Meloidogyne incognita was reduced and seedling growth increased. There are several reports where different oil cakes reduced the population of Meloidogyne incognita on tomato, chillies and tobacco (Gowda 1972, Gowda and Shetty 1973, Trivedi et al., 1978, Desai et al., 1979 and Vijayalakshmi and Goswami 1986).

Oil cakes were equally effective in suppressing the root-knot development and population of other parasitic nematodes on vegetables and perennial crops (Khan et al., 1966, 1973, 1974 a,b, 1979; Alam and Khan, 1974; Alam et al., 1977a,c, Siddiqui et al., 1976) Khan et al., (1976) and Alam et al., (1977a) found that the oil cakes of castor, mustard, margosa and groundnut reduced the population of plant parasitic nematodes in nurseries of grewia, papaya, pomegranate, mango, black berry, lemon, bougainvillea and rose.

Oil cakes when applied in soil in the nurseries of vegetables like tomato, egg plant and chilli also had similar effect on nematode population, thus reducing cost of applicatior (Alam 1976). Alam et al., (1977d, 1980), Mahmood and [illegible] Saxena (1985) demonstrated that the plants grown in oil cake amended soil had higher conc. of phenols probably responsible for some resistance against the attack of Meloidogyne incognita ,

Tylenchorhynchus brassicae and R. reniformis. In order to understand the mechanism involved in the control of nematodes by oil cake amendments some studies have been made. Water extracts of oiled and deoiled cakes and their distillates were found to be toxic to different plant parasitic nematodes, (Khan et al., 1966, 1974b; Rao and Prasad, 1969; Deshmukh and Prasad 1969; Mishra and Prasad 1973; Sitaramiah et al., 1974; Pillai et al., 1974 and Alam et al., 1982). Oil cake extracts of mustard, taramera, Sasame and cotton prevented hatching of eggs of Meloidogyne incognita (Inderjit et al., 1980).

In Heterodera avenae least number of larvae hatched in normal concentration (N) and in 5% of the amendments with oil cakes. Moreover, these amendments significantly reduced the final cyst population and also increased the number of tillers and shoot weight (Sharma et al., 1981). Bhatnagar et al., (1978) pointed out that groundnut 'til' and mustard oil cakes resulted in considerable reduction in root-knot galling. Miller (1979) claimed that some vegetable oils such as corn, cotton seed, linseed, olive or safflower, when incorporated into soil generally reduced the population of P. penetrans. Prasad et al., (1984) tested six chemical pesticides in addition to mustard oil, gingelly oil, sunflower oil and an extract of Eclipta alba for control of Meloidogyne graminicola on rice. All root dip treatments except mustard oil and gingelly oil gave a significant reduction in adult females, egg masses and number of galls. Significant increase in shoot height was associated

with treatment with pesticides and extract of E. alba. Soil drench treatments using pesticides or E. alba extract reduced egg mass and gall number.

Plant parts and plant extracts

The use of plants and plant products has been advocated for the control of plant parasitic nematodes by several workers. The amendments itself may have toxic principles as has been shown by nematicidal properties of water extracts of Anagallis arvensis (Nene and Thapaliyal 1966) Ericeron linifolius (Nene and Kumar 1967), Helenium hybrid (Gommers, 1971), ginger, garlic, chilli and pepper (Sukul et al., 1974). Urtica urens and Cephaloria syriaca (Mohammad et al., 1981). Argemone mexicana and garlic (Nath et al.,1982 a,b), Digitaria decumbans (Haroon and Smart Jr. 1983),Tagetes patula (Rajvanshi et al., 1985)against plant parasitic nematodes. Tiyagi et al., (1985) have reported nematicidal nature of water extracts of different plant parts of some members of the family Compositae. Hatching of eggs of Meloidogyne incognita was inhibited by incorporating alfalfa and soyabean to soil (Johnson and Shamiyeh 1975). There was mortality of larvae in the extracts of, Karanj cake, neem cake and groundnut cake (Desai et al., 1979) and of Meloidogyne javanica in the aqueous extracts of Margosa cake (Sitaramaiah and Singh 1977); and of Meloidogyne incognita and P. penetrans in the extracts of rye and timothy plant residues decomposing in soil (Sayre et al., 1965); and of Tylenchorhynchus dubius and Hoplolaimus sp. by leaves and stem extracts of bean and

leaf extracts of tobacco (Miller et al., 1973). In the extracts of Peristrophe bicalyculata and Acanthocephalus kadamba the mortality of Meloidogyne incognita was determined every 10 min for 2 hours. The petroleum ether extracts of Acanthocephalus kadamba and Tragia involucrata were the most nematicidal followed by the chloroform extract of P. bicalyculata (Chatterjee and Sukul 1980). When aqueous extract of neem leaves was applied to young chickpea plants grown in Meloidogyne javanica infested soil there was significant increase in growth of plant and reduction in root galling. The efficacy however decreased with the decrease in the rate of application (Ram and Gupta 1980). Mohammad et al., (1981) reported that the extracts of leaves of Delphinium ajacis, Urtica urens and Eminium intortum, flowers of Papaver rhoeas, fruits of Citrullus colocynthis and Xanthium strumarium and seeds of Paganum harmala, Brassica arvensis, Lepidium draba and Cephaloria syriaca exhibited nematicidal properties against Tylenchulus semipenetrans when tested in vitro. Leaf extracts of U. urens and seed extracts of C. syriaca were highly effective. Leaf and seed extracts of twelve indigenous medicinal plants adversely affected Rotylenchulus reniformis and Meloidogyne incognita. The efficacy, however, increased with increase in concentration of extracts. (Mahmood et al., 1982). Extracts of leaves of Anagallis arvensis and the seed of Linum usitatissimum and Sida cordifolia proved to be highly toxic. Similar studies have been made with extracts of other plant species (Nandal and Bhatti, 1983, 1986, 1987; Indrarajvanshi et al., 1986; Mani et al.

1986; Rao et al., 1986; Al-obaed et al., 1987; Scramin et al., 1987). Hasan and Jain (1984) while determining the effect of aqueous extracts of fresh leaves, stem and roots of Parthenium hysterophorus (1 : 25, 1 : 50 and 1 : 100) against Meloidogyne incognita and Helicotylenchus dihystera observed 100% mortality in the lowest conc. (1 : 50) of leaf extract, after 25 and 48 hrs. of exposures. Five propenyl phenols viz. chavicol, chavibetal, allylpyrocatechol, chavibetol acetate and allylpyrocatechol diacetate isolated from leaves of Piper betel were found to be of great nematicidal value. Exposure to these compounds resulted in complete mortality of Cenorhabdites elegans. Presence of high concentrations of these compounds in the leaves probably lead to their nematicidal value (Evans et al., 1984). Extracts of plant of Portulaca oleracea, Thymus serphyllum, Coriandrum sativum, Martricaria chamonilla, Peristropha bicalyculata, Tragia involucrata, Anthocephalus kadamba, Ocimum sanctum, O. basilium, Moringa pterygosperma, Tagetes erecta, Mimosa pudica, ginger, chilli, pepper and various other plants significantly affected the hatching of larvae and root gall index caused by Meloidogyne incognita (Abivardi, 1971; Sukul et al., 1974; Mukherjee and Sukul, 1978; Hoan and Davide, 1978; Chatterjee and Sukul 1980, 1981 and Chatterjee et al., 1982). Egunjobi and Afolaimi (1976) pointed out that neem leaf extract significantly reduced the root populations of Pratylenchus brachyurus under semifield conditions. Mohandas et al., (1981) noticed that in rice field heavely infested with *Hirschmanniella* sp. the weed Sphenoclea

zeylanica gave 99% control within 8 weeks. The whole plant and shoot extracts were toxic to nematode after 48 hrs. exposure at 50 g plant tissue/100ml water. Root extracts, however, were not toxic. Haseeb et al., (1982) observed that the aqueous extracts of Mentha viridis, Emblica officinalis and Carissa carandus were toxic to Meloidogyne incognita, while Mentha viridis, Cassia fistula, Cordia myxa, Carrissa carandus, Clocassia antiquorum and Dalbergia sissoo to Rotylenchulus reniformis. Methanol extracts of the roots of Erigeran philadelphicus when fractionated gave two oily fractions with molecular formula of C_{11} H_{10} O_2. These compounds as well as naturally occuring acetylene compounds had high nematicidal activity specially against Pratylenchus coffeae with (50% mortality at doses less than 3 mg/l). Siddiqui and and Alam (1987) reported that when seedlings of tomatoes, eggplant and okra raised from seeds treated with water soluble extracts of Azadirachta indica and Melia azedarach were inoculated with Meloidogyne incognita or Rotylenchulus reniformis the development of galling and the population of R. reniformis were significantly reduced. Goswami and Vijaylaxshmi (1986a, b) tested different plant extracts and oil-cakes against Meloidogyne incognita and found significant reduction in root galls and population of nematode. The water and methanolic extracts of leaves, stems and buds of Datura stramonium, Ipomoea carnea, Tagetes patula and Lawsonia alba were found toxic to second stage juveniles of Tylenchulus semipenetrans and Anguina tritici resulting in their mortality (Kumari et al.,1986)

Salem and Osman (1988) showed that intercropping Tagetes sp. with tomato plants was more effective than adding natural root extracts of Tagetes to the soil in reducing nematode population. Siddiqui and Saxena (1987) pointed out that interculture of tomato and aubergines with Azadirachta indica and Melia azedarach reduced the rate of multiplication of Meloidogyne incognita and Rotylenchulus reniformis together with reduction in root galling. The Quassinoid fraction of the seeds of Hannoa undulata adversely affected the penetration of larvae of Meloidogyne javanica into tomato root and the multiplication (Prot and Kornprobst 1983, 1985).

Amongst different grasses used as cover crops Festuca arundinacea markedly reduced the population of nematodes. Of the different grasses F.arundinacea and F. rubra were more effective in suppressing nematodes than Poa pratensis and Dactylis glomerata (Townshend et al., 1984). Latices of some plants were found highly toxic to plant parasitic nematodes, though to varying extent. The toxicity increased with an increase in the concentration of latex and exposure period. Hatching of the root-knot larvae was also reduced by the latex (Haseeb et al., 1984, Zurreen and Khan 1984, Siddiqui et al., 1984). Singh (1983) reported that culture filtrates of some of the fungi from the rhizosphere of plants grown on amended soil such as Alternaria humicola, Aspergillus niger, Curvularia lunata, Sclerotium rolfsii, Trichoderma lignorum and Trichoderma viride were found to be nematoxic and inhibited the hatching of larvae of Meloidogyne incognita. Haq et al., (1985) observed

considerable reduction in population of Meloidogyne incognita and increase in growth of tomato when fly ash was added in soil.

Integrated pest management approach

Integrated pest management involves reduction of nematode numbers to tolerance level by using a variety of techniques such as predators, parasites genetic resistance, modification of environment and if necessary pesticides in combination. In this direction considerable work has been carried out and the literature is reviewed by Bird (1987). The efficacy of nematode control is increased manifold in this way.

The population of Meloidogyne incognita was found to be reduced significantly alongwith an increase in plant growth and crop yield by integration of different methods of control such as chicken manure and aldicarb; marigold, aldicarb and compost (Ruelo 1983); chicken manure compost, a nematode trapping fungus (Arthobotrys oligospora) and Tagetes patula used in combination and with low doses of aldicarb (AVRDC 1981); carbofuran and oil-cakes together with Zea mays var. seneca, tomato and chilli in rotation (Sundaresh et al., 1977).

Carbofuran, fensulfothion together with sawdust + NPK fertilizers significantly reduced the population of M. javanica (Sitaramaiah and Singh 1976). A combination of neem leaves,

potassium and/or aldicarb gave the significant reduction in gall numbers caused by M. javanica (Ram and Gupta 1982).

Green manuring with sannhemp (Crotalaria juncea) following bajra (Pennisetum typhoides) and application of DBCP prior to tobacco planting reduced the population of Meloidogyne sp. (Patel et al., 1978).

Hot water treatment, neem cake application and phosphamidon were evaluated against Radopholus similis and was found effective in reducing the nematode population and improving plant growth (Ravichandra and Krishnappa 1985). Badra and Elabary (1978) noticed that inorganic fertilizers in the form of N or P with organic amendments gave significant reduction in the population of Rotylenchulus reniformis as well as improving the plant growth. Multiple treatments involving aldicarb and possibly oxamyl alongwith pigeon droppings or poultry droppings increased the growth of tomato and at the same time reduced the population of Rotylenchulus reniformis (Badra and Mohammad, 1979).

For the control of plant parasitic nematodes, Alam and Ashraf (1986) used compost, bone meal, oil seed of castor, mustard, margosa/neem and pea nut, DD, DBCP phorate, fensulfothion and dimethoate, separately and in combination and they observed that organic fertilizers and compost did not

suppress the population of plant parasitic nematodes significantly, while all the other soil amendments and nematicides significantly reduced nematode population; when applied in combination as compared to soil in these treatments alone.

Plan of work

1. To screen wild plants of the family Labiatae, Acanthaceae, Solanaceae, Asclepiadaceae as organic amendments on the multiplication of nematodes in the soil. Following studies will be made on these showing highest efficacy in reduction in nematode population.
2. To determine the optimum concentration of organic amendments.
3. To find out the waiting period required for getting highest reduction in the population of nematode.
4. To work out amount of irrigation required for quick decomposition of organic amendments.
5. To study the effect of different ratios of sand and clay on the efficacy of organic amendments.
6. To determine the organic matter available in the soil as a result of organic amendments.
7. To determine the changes in Bulk density, Real specific gravity and porosity of the soil as a result of organic amendments.
8. To study the changes in pH of the soil during decomposition of organic matter.
9. To integrate different organic amendments with nematicides such as carbofuran.

MATERIAL

&

METHOD

MATERIALS AND METHODS

3.1 Wild plants belonging to the family Labiatae, Acanthaceae, Solanaceae, Asclepiadaceae will be collected from the field and brought to the laboratory. These plants would be thoroughly cleaned and washed and would be chopped into small pieces. These organic amendments will be added to naturally infested soil in the pots @ 5g/100g of soil. Population of plant parasitic nematodes before adding the organic amendments and after would be determined. On this basis the reduction in population of key pests i.e. root-knot nematode, reniform nematode and stunt nematode will be determined. Plants exhibiting moderate to highest reduction in nematode population will be selected for further study.

3.2 In order to determine the optimum concentration required for the reduction in the nematode population the soil will be amended with different amounts of organic amendments of the plants selected above i.e. 5, 10, 15, 20 g per 100 g of soil. Population of nematodes will be determined after 5, 10 and 15 days to determine the duration at which highest reduction is obtained.

3.3 The soil will be amended with optimum amount of the organic amendment. Seedlings of tomato cv. Pusa Ruby raised in the nematode free soil will be transplanted after 5, 10, 15 and 20 days of amending the soil. The

seedling will later be inoculated with 1000 larvae of root-knot nematode Meloidogyne incognita from the cultures maintained in the department. After 45 days of inoculation plants will be uprooted. Growth of plants and root-knot development will be determined. This study will help in understanding at which stage of decomposition of the organic amendments the phytotoxicity is minimum.

3.4 Before concluding the efficacy of any organic amendment it is essential to work out amount of water required for decomposition of organic matter, the pots amended with organic amendment will be irrigated daily, on alternate days and twice a week during winter during summer. After the waiting period the seedling of tomato cv. Pusa Ruby will be grown and inoculated with the larvae of root-knot nematode. After 45 days the plants will be uprooted, growth of plants and development of root-knot will be determined.

3.5 Efficacy of an organic amendment is also influenced by the type of the soil, therefore attempts will made to determine the effect of different ratios of sand and soil on the efficacy of the organic amendments. Mixtures of the sand and clay will be made in the ratio of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9. These soil contained in the pots will be amended with organic amendments in the optimum concentration and will be giving optimum decomposition period and optimum amount

of water. Seedling of tomato cv. Pusa Ruby will be transplanted in these soils and will be inoculated with root-knot nematode. Growth of plant and development of root-knot will be determined after 45 days of inoculation.

It is likely that organic amendment in different combination may be more effective. Therefore, plants showing efficacy in reducing nematode population will be rated as very good, good/moderate. Different combination of these organic amendments will be made. The soil contained in the pots will be amended with organic amendments of the above combinations, with optimum dose and will be given optimum waiting period. Seedlings of tomato cv. Pusa Ruby will be transplanted and inoculated. Growth of plants and development of root-knot will be determined. These will also be integrated with carbofuran at the rate of 10kg/ha.

3.6 Soil organic matter will be determined in natural soils and in amended soil by using Walklay and Black rapid titration method, (Walklay and Black, 1947).

The soil will be digested with chromic and sulphuric acid making use of the heat of dilute of sulphuric acid. The excess of chromic acid not reduced by the organic matter of the soil will be obtained by titrating with standard ferrous ammonium sulphate solution. The following reagents will be prepared.

i) Potassium dichromate 1 N :- 49.0 g of $K_2 Cr_2 O_7$

will be dissolved in water to make it to one litre.

ii) Sulphuric acid not less than 98%.

iii) Phosphoric acid 85%.

iv) Diphenylamine indicator solution: of Diphenylamine (0.5g) will be dissolved in a mixture of 100 ml sulphuric acid and 20 ml water and will be stored in a coloured bottle.

v) Ferrous ammonium sulphate: (N/2) will be obtained by dissolving 392.0g Fe SO_4 $(NH_4)_2$ SO_4 $6H_2O$ (AR) in water. To this will be added 15 ml sulphuric acid. It will then be diluted to 2 litres.

Soil (2-10g) (0.5mm) will be taken in a 500 ml conical flask and 10 ml of 1N potassium dichromate solution and 200 ml of concentrated sulphuric acid will be added. This would be shaken for a minute or two and will be allowed to stand on an asbestos mat, for about 30 minutes. Later 200 ml of water, 10 ml of phosphoric acid and 1 ml of diphenylamine indicator solution will be added. A deep violet colour will appear. This will be titrated with N/2 ferrous ammonium sulphate solution, till the violet colour changes to purple and finally to green.

In the same way a blank titration will be carried out and the amount of organic matter will be calculated

by using the formula:

$$\text{Percentage of organic carbon in the soil} = \frac{(x-y) \times .003 \times 100}{2 \times W} = Z$$

and $$\text{Percentage of organic matter} = Z \times 1.724$$

Where x is the volume of ferrous ammonium sulphate required for reducing 10 ml potassium dichromate solution, y is the volume of ferrous ammonium sulphate required for reducing excess of potassium dichromate W is the weight of soil taken fo analysis and 1.724 is the Walklay Black value and 0.003g carbon present in 1 ml of normal $K_2 Cr_2 O_7$.

3.7 The Bulk density, real specific gravity and porosity of a soil will be determined by the standard technique in both natural and organic matter amended soil.

The bulk density of soil is the mass of the soil per unit volume and porosity of soil is the fraction of soil volume not occupied by soil particles. Bulk density of most soils ranges from 1.02 to 1.8 g/cc.

A bottle of 50 ml will be weighed and filled with soil up to the brim tapping the bottle about 20 times so that it is completly filled. It will be weighed again, the soil will be removed from bottle. The bottle will be filled with water. The amount of water required to fill the bottle will be measured. The apparent or bulk density will be obtained by dividing the weight of the soil with volume of the soil.

Calculation:

Weight of empty bottle = W_1 g

Weight of bottle and soil = W_2 g

Weight of soil = $W_1 - W_2$

Volume of the soil or volume of water needed to fill the bottle = V ml

Bulk density = $\frac{W_1 - W_2}{V}$ g per cm^2

After calculating bulk density and real specificity, the porosity of the soil can be calculated by formula:

$$\text{Pore space} = \frac{100(\text{true specific gravity} - \text{apparent specific gravity})}{\text{True specific gravity}}$$

3.8 pH of the soil was determined in 1:2 or 1:5 soil suspensions by using Elico pH meter. (Jackson 1958).

3.9 Raising seedlings and Inoculation with nematodes

Seedlings of tomato cultivar Pusa Ruby will be raised in nematode free soil in the green house. Before sowing, the seeds will be surface sterilized with 1:1000 mercuric chloride and rinsed with sterilized distilled water. Seedlings when 3 weeks old will be used for transplantation. In order to prepare mercuric chloride solution 1g of mercuric chloride will be dissolved in the least amount of concentrated hydrochloric acid required for dissolving the crystals. To this 100 ml of sterilized distilled water will be added and this would be labelled as stock solution. To this 10 ml stock

solution 90 ml of sterilized distilled water will be added to obtained the concentration of 1:1000.

In order to make the soil nematode free, carbofuran @10kg/ha of the soil will be added and after giving a waiting period of 15 days it will be used.

Inoculum will be raised from single egg mass culture of Meloidogyne incognita maintained in the microplots. In order to obtain larvae for inoculation, freshly collected egg masses will be made to hatch in distilled water. When the larvae hatch out larval suspension will be made in distilled water containing 1000 larvae/10 ml of the suspension. For inoculation near the seedlings four holes will be made and the nematode suspension would be added by Pipette.

Isolation of nematodes from the soil will be made by using Cobb's sieving and decanting method. Soil (200g) will be placed in plastic pan and to this will be added 1 litre water. After soaking and breaking any lump gently with the fingers, stones will be removed. The soil will be stirred and allowed the heavy soil particles to settle to the bottom for about 5 seconds. This suspension will be poured through seive of 15 mesh pore size into a second pan. Some more water will then be added to the residue in first pan. This process will be repeated and the remainder will be discarded. It will be washed out in first pan.

The suspension will then be passed through sieve of 50, 100, 200 and 300 mesh poresize. Each time the debris will be transferred to a 250 ml beaker. All the suspensions will be mixed. The nematodes will be allowed to settle down on the bottom of the beakers, the excess water will be decanted and will be poured over a muslin filter into a Baermann funnel fitted with a rubber tubing closed by a clip. The funnel will be filled with tap water until it touches the muslin filter. After about 24 hrs the nematode suspension will be collected by opening the clip for microscopic examination and counting of the nematodes.

3.10 Growth of plants

Through out the studies growth of plants will be determined after 45 days of inoculation. Plants will be uprooted and washed in running water, length of root and shoot will be determined. The plants will be weighed after putting them in the blotting paper so that extra amount of water is absorbed. This would give the fresh weight of the plant. The plants will be dried in oven at $60^{0}C$ and after being cooled they will be weighed, to get dry weight of a plant. Root-knot index will be determined on 1-5 scales as follows:

i) No gall No egg masses - Highly resistant

ii) 1 to 10 galls/egg masses - Resistant

iii) 11 to 30 galls/egg masses - Moderately resistant

iv) 31 to 100 galls/egg masses - Susceptible

v) 101 and above galls/egg masses - Highly Susceptible

Through out the studies there will be five replicates of each treatment and each experiment will be repeated twice. The data so generated will be subjected to statistical analysis.

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